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Abstract: The most prominent responses of Lake Zurich to climate warming include the increase of surface water temperatures, a reduced depth of spring mixing, and the persistent thriving of the harmful cyanobacterium Planktothrix rubescens, a low-light adapted species concentrating in the metalimnion during summer. To study changes of its habitat, we assessed the spatio-temporal metalimnetic boundaries by applying low-pass filtering and binary thresholding to temperature profiles of long-term data (1978–2013, weekly measurements). Due to increasing temperatures over the last 3.5 decades, the onset and duration of metalimnion formation changed significantly (stratification increased by 33 days). Moreover, the upper metalimnetic boundary has undergone a significant drawdown of 2.3 m, accounting for an overall decrease in metalimnion thickness. Being the most abundant species in this zone, P. rubescens was not negatively affected by altered temporal or spatial stratification patterns during its phase of net growth (July–September), as the zone of its optimal light-dependent buoyancy was still located within the metalimnion. The biomass of P. rubescens in August was even significantly related to increasing temperatures. Nevertheless, a further depression of the metalimnetic top boundary may eventually restrict P. rubescens by forcing it into layers of unfavourable light conditions or into the turbulent epilimnetic zone.

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12 **Abstract**

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27 further depression of the metalimnetic top boundary may eventually restrict *P. rubescens* by
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29

30 Keywords: lake warming, Lake Zurich, long-term data, metalimnion, neutral buoyancy,
31 *Planktothrix rubescens*

Introduction

Lakes are good reflectors of local aspects of climate change by having the characteristics of enclosed ecosystems, and at the same time by being in dynamic exchange with the atmosphere. Many studies of European lakes over the last decades have thus focused on different aspects linked to climatic change. These included changes in thermal stratification and mixing (Livingstone, 2003), oxygen content (Posch et al., 2012; North et al., 2014), nutrient loading (Straile et al., 2003; O'Neil et al., 2012) and phytoplankton response (Weyhenmeyer et al., 2002; Winder & Sommer, 2012). One of the most important issues related to some of these changes is the large-scale occurrence of harmful algal and cyanobacterial blooms (Johnk et al., 2008; Paerl & Huisman, 2008; Paerl et al., 2011).

The response of Lake Zurich (Switzerland) to climatic conditions has been extensively investigated. It has been shown that warm winters have a positive effect on thermal stability, and a negative impact on the spring mixing depth (Dokulil et al., 2006; Rempfer et al., 2010; Posch et al., 2012). Livingstone (2003) has reported on the long-term increase of mean water temperatures, on the prolongation of lake stratification and on the relationship between climate warming and the reduced occurrence of complete water turnover (holomixis).

The most abundant primary producer in Lake Zurich is the toxic filamentous cyanobacterium *Planktothrix rubescens* (Bossard et al., 2001). *P. rubescens* is a major part of the phytoplankton community also in numerous other European lakes, including, e.g., Lake Mondsee, Austria (Dokulil & Teubner, 2012), the Italian lakes Garda, Iseo and Como (Salmaso et al., 2003; Salmaso, 2010), Lake Ammersee, Germany (Ernst et al., 2001), and until recently, Lake Bourget, France (Jacquet et al., 2014). The present dominance of *P. rubescens* in Lake Zurich was established during the re-oligotrophication period in the late 1970s (Supplementary Material, Fig. S1), characterised by reduced nutrient loadings (Gammeter & Zimmermann, 2001) and severely changed nutrient stoichiometry (Posch et al., 2012). Since then *P. rubescens* has been forming persistent reoccurring blooms of increasing

biomass, representing a potential hazard for humans and animals due to highly toxic secondary metabolites such as microcystins (Blom et al., 2006).

This cyanobacterium is adapted to low light intensities, and, therefore, it is forced to concentrate in the metalimnion (10-15 m) during the warm seasons (Supplementary Material, Fig. S1). As long as there is a stable thermal stratification, the metalimnion as a habitat guarantees saturating light conditions for cyanobacterial growth, and hampers the entrainment of cyanobacteria into the turbulent epilimnetic zone. During summer, high irradiance in the epilimnion would have a strong negative impact on this low-light adapted organism. *P. rubescens* can regulate its vertical position in the water column, and buoyancy is provided by intracellular gas vesicles, which allow a depth adjustment of 0.4 m d^{-1} (Walsby et al., 2001). Thus, cyanobacteria can actively concentrate in the metalimnion during thermal stratification. The critical collapse pressure of the gas vesicles is reached at approximately 80-90 m depth, below which they are destroyed (Walsby et al., 1998). This aspect becomes of relevance in spring, when holomixis transports cyanobacterial filaments into the deep hypolimnion. The successful establishment of *P. rubescens* in many lakes is in large parts attributed to climate warming: due to stabilisation of the thermal stratification and the reduced mixing depth, *P. rubescens* loses less biomass during warm winters (Walsby et al., 1998; Posch et al., 2012).

Even though the characteristics and development of *P. rubescens* in Lake Zurich are well studied, little is known about how climate warming influences its very habitat during stable thermal stratification, i.e., the metalimnion, and if such changes potentially affect *P. rubescens* population dynamics. In this study we used available long-term data of cyanobacteria and physicochemical parameters to test the following three theses: (i) Increasing water temperatures due to climate warming will affect the temporal and spatial dynamics of the metalimnion in Lake Zurich; (ii) The expected earlier onset and temporal extension of the metalimnion period is significantly related to the increasing biomass of *P.*

rubescens; (iii) Changes in the spatial dimensions and of other physical properties of the metalimnion affect *P. rubescens* during its growth period in summer.

Materials and Methods

Study site

Lake Zurich (also referred to as Lower Lake Zurich) is a large (65 km^2, 30 km length), deep (136 m), stratifying, prealpine lake located on the Swiss Plateau. It is well separated from the fairly smaller Upper Lake Zurich by a sill, allowing for the inflow of only the upper 3 m of surface water, and which is nevertheless the major inflow of Lake Zurich. The thermal regime of the lake can be classified as generally monomictic with a water turn-over in early spring. The lake undergoes holomixis after cold winters, whereas partial mixing (80-100 m) occurs after mild winters (Posch et al., 2012). In late autumn, thermal stratification of the upper water body gets eroded, which leads to a turbulent mixed zone between 0-30 m. Lake Zurich is an important freshwater reservoir for over 1 million people. Over the past century the lake has been exposed to regular monitoring and management, involving a re-oligotrophication process with reduced phosphorus deposition starting in the 1950s, improved waste water treatment, inflow and outflow control. For further limnological information and a map of the lake we refer to Garneau et al. (2013).

Long-term data

Data of water temperature and photosynthetically active radiation have been collected weekly near the deepest point of Lake Zurich (N 47° 17.147, E 8° 35.460; 406 m a.s.l. at surface) for the period of 1978 to 2013. In total, 1517 temperature profiles (0-20 m, unless otherwise stated) were measured at 1 m intervals using a TTM 72 thermistor thermometer (Züllig AG, Rheineck, Switzerland) between 1978 and 2008, and a YSI multiprobe (Yellow Springs Instruments 6600) from 2008 onward. Underwater light profiles were determined with a

spherical quantum sensor (LI-Cor) for the period of 1985 to 2013 at the same spatial and temporal resolution as temperature measurements. A dataset of monthly *P. rubescens* biomass profiles (0, 1, 2.5, 5, 7.5, 10, 12.5, 15, 20, 30, 40, 80, 120, 135 m), measured also at the deepest point of the lake (136 m) between 1972 and 2011, was provided by the City of Zurich Water Supply Company (Supplementary Material, Fig. S1). Air temperature analysis for the corresponding study period (1978-2013) is based on monthly averages provided by the Federal Office of Meteorology and Climatology MeteoSwiss measured in Zurich-Fluntern (N 47° 22.7, E 8° 33.9; 556 m a.s.l.). The meteorological station is located in a distance of 10 km north of the deepest point (sampling point), and 2.8 km from the northern end (major outflow) of Lake Zurich.

Determination of the boundaries and duration of the metalimnion

The metalimnion of lakes is defined as the zone with the steepest thermal gradient (usually Δ 0.5 - 4.5 °C m^{-1}), thereby forming a physical barrier for mixing between surface and deep water layers. Its formation in spring, caused by the progressive warming of surface waters, can be described as a dynamic process. Sudden cooling events in this time of the year often lead to a disruption of the metalimnion followed by re-stabilization. This makes the determination of an exact time point of metalimnion formation especially challenging. We compared two different years to illustrate this problem (Fig. 1). To define spatial and temporal boundaries of the metalimnion, the two dimensional (time, depth) temperature profiles were low-pass filtered with a linear kernel (size = 3 × 3) to remove extreme transitions between sampling points and short timed effects. Subsequently, the coherent area which covered a maximum of data points with a temperature gradient of ≥ 0.5 °C m^{-1} was defined as the metalimnion (Fig. 1). The definite starting and ending dates were determined by linear interpolation between the adjacent weekly sampling dates. The duration of the metalimnetic stratification was determined as the difference between the last and the first date (in Julian

day) of metalimnion formation for each year. Metalimnion thickness was defined as the difference between the lower and the upper depth boundary, and its center was calculated as the mean of these boundaries.

Data treatment

Serial correlation of the residuals of time series can significantly affect trend estimates (von Storch, 1995), thus it has to be eliminated. For each set of monthly averaged air and water temperatures, as well as metalimnion spatial and temporal data we applied the "trend-free pre-whitening" (TFPW) procedure proposed by Yue et al. (2002). This method first evaluates the presence of a trend by assessing if the trend slope *b* is close to zero (no trend) or not (assumption for a linear trend). If a trend is detected, it is subtracted from the data. Significant autocorrelations at lag 1 of the detrended series are then removed. Subsequently, the trend is re-added to the pre-whitened series. Following this, adjusted Mann Kendall (MK) *tau* coefficients, *p*-values and Sen's slopes (Sen, 1968) of the autocorrelation-free data are estimated. The overall trends in air temperature, as well as trends of water temperature for each depth (0-20 m) over the whole study period (1978-2013) were analyzed with MK seasonal trend tests due to the presence of strong reoccurring annual patterns. Serial dependence was taken into account according to Hirsch & Slack (1984).

The magnitude of significant changes in the studied parameters over time was calculated using the linear regression equation $Y = b \cdot X + a$, with Y being the predicted value (e.g., temperature), *b* the Sen's slope of the regression line, X the predictor (e.g., year), and 'a' the intercept of Sen's slope. Based on this equation we determined the Y values for the years X=1978 and X=2013, and calculated, e.g. the temperature increase, as the difference between Y_{2013} and Y_{1978}.

159 Spearman's correlation test was used to test for significant correlations between *P.*
160 *rubescens* biomass and metalimnion spatial and temporal dimensions, as well as between
161 biomass and neutral buoyancy depth.
162 Linear regression was applied to assess the relationship between the biomass of
163 metalimnetic *P. rubescens* populations in summer, as well as between these populations and
164 the temperature of metalimnion center. The residuals from the linear model were tested for
165 normality and significant serial dependence using the autocorrelation- and partial
166 autocorrelation functions. No significant autocorrelations were detected for this data.
167 Schmidt stability is defined as the energy per unit area required for mixing without
168 input or loss of heat in lakes (Idso, 1973; Schmidt, 1928). For a comparison with our
169 metalimnion determination approach, we estimated Schmidt stability for each temperature
170 profile only for the zone of 0-20 m, which corresponds to the epi- and metalimnion in Lake
171 Zurich. Estimation of the energy required to mix the whole water column down to 136 m, as
172 already shown by Livingstone (2003), would give information about potential full turn-over,
173 which is outside the scope of this study. Furthermore, in the last decade the mixing depth in
174 Lake Zurich has rarely exceeded 130 m (Posch et al., 2012). Stratification start and end points
175 were estimated based on several Schmidt stability thresholds (10-150 J m^{-2}) for each year of
176 the period 1978-2013, after linear interpolation of calculated values.
177 *P. rubescens* biomass (*BPr*, in µg L^{-1} fresh weight) was first linearly interpolated to
178 achieve a vertical resolution of 1 m intervals. The total biomass (in tons) of a depth layer (z)
179 was calculated by multiplying BPr_z with the total water volume of this layer (V_z, in m^3). Total
180 epilimnetic biomass (in tons) was calculated with the following equation:

181 $$Total\ epilimnetic\ biomass\ (tons) = \sum_{z=0}^{20} BPr_z \times V_z$$

182 The water volume for z = 0 m (V_0) comprised the surface layer from 0 m to 0.5 m. For all
183 other water layers (z = 1-20 m), volumes for a defined depth z comprised the zone of 0.5 m

above and below z (e.g., V_1 = water volume between 0.5-1.5 m). The annual population minimum of *P. rubescens* in June also represents the start of its seasonal growth period, which ends with the annual population maximum in November.

Neutral buoyancy depth was defined after Walsby et al. (2004) as the depth with an irradiance level at which 50 % of *P. rubescens* filaments float, i.e., 6.51 µmol m^{-2} s^{-1}. This depth was estimated by cubic spline interpolation of the underwater light attenuation curves.

Data interpolation, statistical analyses and Schmidt stability calculations were performed using the software R. TFPW was computed using the package 'zyp'. For MK seasonal trend test we chose the package 'EnvStats'. Schmidt stability was calculated using the package 'rLakeAnalyzer'. Bray-Curtis dissimilarity indices were calculated with the add-in software XLSTAT (Addinsoft™) in Excel.

195 **Results**

196 **Long-term trends in air temperatures for the period 1978-2013**

197 MK trend tests for discrete months showed highly significant increases in air temperatures for
198 the spring months (April: *tau* = 0.44, $p < 0.001$; May: *tau* = 0.33, $p < 0.01$; June: *tau* = 0.36, p
199 < 0.01, $n = 36$ for each month) and for November (*tau* = 0.27, $p < 0.05$, $n = 36$). Air
200 temperatures in all other months showed positive trends (except for December) but no
201 statistically significant time trends (Fig. 2, Supplementary Material, Fig. S2). Nevertheless,
202 overall air temperature for the same period increased significantly by 1.5 °C (seasonal MK:
203 *tau* = 0.2, $p < 0.001$, $n = 432$; Fig. 2). The most pronounced rise in air temperature was in
204 April, May, and June (3.2 °C, 2.4 °C and 2.5 °C), and these months predominantly accounted
205 for the overall trend. The alternating occurrence of colder and warmer years appeared to
206 interfere with any long-term trend of temperature during the winter months (December to
207 February; Supplementary Material, Fig. S2).

208

209 **Thermal characteristics of the water column**

210 In parallel with air temperature trends, the water column of Lake Zurich exhibited distinct
211 changes in thermal structure. Local air temperature dynamics are closely linked to changes in
212 the thermal properties of surface layers in lakes (Livingstone, 2003; Straile et al., 2003;
213 Dokulil, 2014).

214 An in-depth analysis of the long-term monthly averages per depth layer did not only
215 reveal a significant temperature increase in the upper 5 m of the water column in several
216 months, but also a large-scale, yet uneven, warming pattern across the whole upper water
217 column (0-20 m, Fig. 2). In contrast to air temperature, where significant trends were only
218 observed for four months, water temperatures significantly increased from March until
219 December at certain depths. In spring (April to June), surface layers showed significant
220 temperature increase, affecting the water column even down to 14 m in June. From July on,

lake warming was restricted to deeper layers, and in October significant trends could be only determined for the water column between 10 and 15 m (Fig. 2). In November a warming of the surface, and from 4-17 m depth was observed, which reflects both, the increase in air temperature and the erosion of the epi- and metalimnetic stratification. As the significant air temperature increase in November seemed to affect only the surface, a possible heat transfer from the former metalimnion into surrounding water layers is very likely. The lack of significant air temperature increase in December contradicts the water temperature increase in 0-20 m observed in this month, which could also be an indication for distribution of heat from deeper layers.

Trend analyses calculated per depth and for the full year showed a homogenous warming of the surface water body between 0 to 15m during the last 36 years (Fig. 2, overall trends). Overall, the water temperature increased by up to 1.4 °C in 7-9 m (seasonal MK, each: $tau = 0.3$, $p < 0.001$, $n = 432$).

The maximum increase per depth on the surface (0-4 m) was found in May (Fig. 2, right panel). With increasing depth (4-12 m) maximal increases were observed in July and August. The strongest rise in water temperature per depth were recorded for July in 6 m and 7 m depth (max. 4.13 °C for both; Fig. 2, right two panels). In deeper layers maximal temperature rises were recorded in October (13-15 m) and December (16-17 m).

Additionally, the increase in water temperature seemed to be more pronounced than that in air temperature over time. A comparison between the Sen's slopes of water and air temperatures for each month and depth revealed higher values of the former at several depths in May and June (Supplementary Material, Fig. S3).

Temporal changes of metalimnion dynamics

The metalimnion determination highlighted significant changes of temporal dimensions in Lake Zurich (Fig. 3). Onset and duration of metalimnion appearance showed significant

trends over the total period (Fig. 3a). Stratification onset denoted a preponed shift of 17 days (MK: *tau* = -0.32, $p < 0.01$, $n = 36$), whereas stratification end exhibited no significant trend between 1978 and 2013. Duration of metalimnion appearance, however, was extended by 33 days over the study period (MK: *tau* = 0.36, $p < 0.01$, $n = 36$; Fig. 3b).

The constant shift towards an earlier onset propagated clearly through decades. In the first decade, 75 % of metalimnion formation started in May, and 25 % in April. In the second decade, 60 % of onsets occurred in April, and 40 % in May. During the last decade, 43 % of metalimnion onsets were in April, 43 % in May, and 14 % already in March. In addition, 63 % of the years with duration > 200 days were found in the third decade, whereas 75 % of the years with shortest duration (< 170 days) fell into the first one.

Comparison of metalimnion detection and Schmidt stability

Onset, end and duration of the metalimnion for each year were compared to equivalent values of annual stratification stability determined by Schmidt stability analysis with thresholds ranging between 10 and 150 J m^{-2} (Supplementary Material, Fig. S4). The best agreement between the two approaches was found at a threshold of 110 J m^{-2} for onset and duration (R^2 > 0.7). The agreement between Schmidt stability and metalimnion end were considerably lower (R^2= 0.3) and comparable results were achieved with thresholds between 30-110 J m^{-2}.

Spatial dynamics of the metalimnion

Analyses for each month during metalimnion appearance showed that in July and August, the top boundary exhibited a significant lowering by 2.3 m and 1.2 m, respectively (MK, each: *tau* = 0.3, $p < 0.05$, $n = 36$) between 1978 and 2013 (Fig. 4a). The development of the lower boundary showed a significant increase by 2 m (MK, *tau* = 0.4, $p < 0.05$, $n = 28$) in November and no trend for the rest of the year (Fig. 4b).

Metalimnion thickness is determined as difference between its lower and upper boundaries. The metalimnion underwent a significant decrease in thickness by 2.8 m in July (MK, *tau* = -0.4, $p < 0.001$, $n = 36$) and by 1.8 m in August (MK, *tau* = -0.4, $p < 0.01$, $n = 36$) over the studied period (Fig. 4c). By contrast, it exhibited a significant increase in thickness by 2 m in November in the same period (MK, *tau* = 0.4, $p < 0.01$, $n = 28$). The MK trend analysis for average metalimnion thickness in July and August indicated a clear loss of thickness by 2.4 m during the last 36 years (Fig. 4d).

Impact of metalimnion changes on the occurrence of *P. rubescens*

Our thesis that longer metalimnion duration would have a positive influence on *P. rubescens* development could not be verified. There was no significant correlation between the population maximum of *P. rubescens* (tons lake^{-1}) in November and metalimnion duration (Table 1). However, a significant correlation was found between the start population size of *P. rubescens* and stratification onset (Table 1), i.e., years with early metalimnion formations (in Julian days) were also characterized by larger cyanobacterial biomasses already in spring. Thus, shorter water turnovers during the winter months favored the survival of *P. rubescens* populations (Posch et al., 2012). This effect seemed to propagate throughout the season, as we found also a significant correlation between metalimnion onset (in Julian days) and *P. rubescens* biomasses in November (Table 1).

We also assessed possible effects of metalimnion changes on the development of *P. rubescens* during its growth phase. The depth of population maxima (μg fresh-weight L^{-1}) in June was significantly correlated with the neutral buoyancy depth (Spearman's *rho* = 0.49, $p < 0.05$), but not with the boundaries of the metalimnion (Fig. 5, Supplementary Material, Fig. S5). In July the depth of maximum biomass correlated with the center of the metalimnion (Spearman's *rho* = 0.38, $p < 0.05$), and the majority of *P. rubescens* remained within the

297 boundaries of neutral buoyancy. This was also true for the situation in August. In September
298 the depths of both *P. rubescens* maxima and neutral buoyancy shifted toward the upper
299 boundary of the metalimnion (Fig. 5; Supplementary Material, Fig. S5). Regression analysis
300 indicated that the biomass of *P. rubescens* in the metalimnion during the summer months was
301 strongly determined by the biomass of the previous month (July-June: $p < 0.001$; August-July:
302 $p < 0.001$; Fig. 6a,b). In addition, the biomass in August was also significantly related to the
303 temperature of the metalimnetic center ($p < 0.001$; Fig. 6d), where we also observed a
304 significant long-term increase of water temperatures (Fig. 6f). However, these trends were not
305 observed for the month July (Fig. 6c,e).

306 **Discussion**

307 **Impact on climate warming on metalimnion temporal dynamics**

308 So far changes of air temperature have been typically analysed at seasonal, annual or decadal
309 resolution in limnological studies (Livingstone, 2003; Blenckner et al., 2007; Dokulil et al.,
310 2010). A more in-depth analysis, however, shows the contribution of particular months to
311 overall trends. For the region of Lake Zurich we found strongest air temperature increases in
312 spring (April to June) and in November. Increase of air temperature particularly in spring can
313 be crucial for establishing thermal stability and for timing of metalimnion onset in a lake. This
314 is important as a weak thermal stratification can often be disrupted by weather-induced
315 internal waves in spring (Bleiker & Schanz, 1997). It has been shown that air temperature is a
316 reliable forcing factor reflecting climate warming, which can significantly influence the heat
317 balance of lakes (Sweers, 1976; Rempfer et al., 2010). Our analysis considered only
318 temperature changes based on links between external warming and lake warming. However,
319 other factors involved in the thermal budget of Lake Zurich, such as heat fluxes via inflows
320 and outflows, and precipitation have been shown to be rather negligible for the total heat
321 balance of the lake (Imboden & Wüest, 1995).

322 We document a clear progression of metalimnion onset in Lake Zurich from May to
323 April and March, respectively, between 1978 and 2013. Similar shifts in onset timing were
324 also found for Müggelsee, Germany (Shatwell et al., 2008). Moreover, the earlier onset rather
325 than the later termination was primarily (significantly) responsible for the observed
326 prolongation of the metalimnion period. This is consistent with results from other studies on
327 stratification dynamics in lakes of the Northern Hemisphere. Winder & Schindler (2004)
328 reported on the importance of an earlier onset for the prolongation of stratification in Lake
329 Washington, USA. Dokulil (2014) also highlighted the important contribution of a shifted
330 onset to the length of stratification in Lake Mondsee, Austria. Both studies reported a
331 prolongation of the stratification by > 25 d, which is in agreement with the overall extension

of 33 d found for Lake Zurich between 1978 and 2013. Our results also confirm the findings of Livingstone (2003), who reported on a stratification extension of up to 3 weeks based on a 50 years data set of Lake Zurich. However, we could not confirm the decisive role of stratification ending for this prolongation, as it was suggested in that study.

The water column in Lake Zurich underwent a significant but uneven large-scale temperature increase during the studied period. The warming of the upper layers in spring and summer subsequently shifted downwards and mainly seemed to affect the metalimnion in September and October. Interestingly, the strongest annual increase in water temperature was found in or close to the metalimnetic zone. Furthermore, water temperature increases in May, July and August partially exceeded even the strongest increase in air temperature found in April (Supplementary Material, Fig. S3). Discrepancy in temperature increase of water versus air associated with reduced lake cooling has also been documented in the surface waters of Lake Superior, USA (Austin & Colman, 2007) during the summer months.

Water temperature increases in November and December were detected for nearly the whole water body (4-17 m and 0-20 m, respectively). However, the significant rise in air temperature in November seemed to affect only the surface (0 m) water temperature in this month. These findings lead us to hypothesize that strong air temperature increase in spring causes an accumulation of thermal energy in the metalimnion. Once a stable metalimnion is formed in late spring/early summer, the storage of heat in the following months (July-September) is rather unaffected by cooling events and turbulent heat exchange (Wuest & Lorke, 2003). Starting from November, when the physical separation between epi- and metalimnion gets eroded due to autumnal surface mixing, a dispersal of thermal energy from the former metalimnion into the mixed zone is very likely.

Comparison of Schmidt stability estimations and metalimnion detection approach

Assessing the spatial development of the metalimnion requires a good resolution of water temperature profiles. Hence, many studies on the specifics of thermal stratification involve modelling approaches (Peeters et al., 2002; 2007), or Schmidt stability estimations (Straile et al., 2003; Rempfer et al., 2010). Schmidt stability exactly defines the stability of the water column based on density gradients, however, it does not inform about the spatial structure of stratification within a lake, i.e. about the division in epi-, meta-, and hypolimnion.

Metalimnion is per definition the zone with the steepest thermal gradients per depth, thus, we based our approach on gradient detection. We applied a technique known from image analysis, two-dimensional linear smoothing (low-pass kernel) followed by binary thresholding and detection of the largest coherent area. This approach overcomes the possible difficulties of estimating metalimnion boundaries due to unstable stratification phases caused by short-term weather changes (Fig. 1). At the same time, this method allowed for a depiction of the metalimnion as a dynamic entity and the assessment of its shape.

In terms of temporal dynamics, our approach of metalimnion detection agrees well with stratification estimations by Schmidt stability, if both analyses were conducted for the upper 20 m. Livingstone (2003) estimated a threshold of ≥ 200 J m^{-2} for stratification onset and ≤ 200 J m^{-2} for stratification termination considering the whole water column in Lake Zurich. Since we limited our analysis to the upper water layers (0-20m), this threshold cannot be applied in our case. We thus conducted a series of analyses with varying Schmidt stability thresholds, which resulted in maximal agreement between both methods at 110 J m^{-2} (Supplementary Material, Fig. S4).

Changing spatial structures of the metalimnion in Lake Zurich

The pronounced lowering of the upper metalimnion boundary (Fig. 4) in summer in Lake Zurich suggests a direct effect of the strong temperature increase in spring, leading to subsequent greater stratification stability and enlargement of the epilimnion. More stable

383 conditions could influence the food web of lakes through enhancing niche separation and
384 providing refuge zones for zooplankton (Teubner et al., 2003; Guilizzoni et al., 2012;
385 Gauthier et al., 2014). Moreover, the deepening of the upper boundary in July and August
386 accounts for the overall decrease in metalimnion thickness over the whole period. The local
387 increase in metalimnion thickness observed in November was most likely related to the
388 prolongation of the stratification, which led to a more pronounced presence of a metalimnion
389 in this month.
390
391 **Context between changing metalimnion dynamics and *P. rubescens***
392 We explored the thesis that *P. rubescens* might be affected by climate warming induced long-
393 term changes of the metalimnion. Recently Posch et al. (2012) showed that the depth of
394 spring turnover has a crucial effect on the subsequent annual population of *P. rubescens* in
395 Lake Zurich. The yearly population growth of these cyanobacteria commences in June and
396 they reach highest concentrations in November. Hence, the magnitude of biomass which
397 survives the cold winter and spring mixis seems to be decisive for the total population size in
398 the subsequent year. Since climate change has altered Lake Zurich mixing regime towards a
399 more frequent occurrence of years with insufficient spring mixis, *P. rubescens* has been able
400 to form larger annual populations with steady increasing biomass over the last years.
401 In addition to these observations, we expected that the trend of increasing metalimnion
402 duration and changes in its thickness might also have an influence on the success of *P.*
403 *rubescens* in Lake Zurich. However, the expanded metalimnion duration does not seem to
404 significantly affect the population maxima of *P. rubescens* found in November. Indeed, the
405 thermal stratification in Lake Zurich occasionally lasts until mid-November. Nevertheless, in
406 October and November *P. rubescens* is distributed over the upper 20 m of the water column
407 as it gets entrained in epilimnetic layers due to instable weather conditions (Van den
408 Wyngaert et al., 2011). Therefore, the presence of the metalimnion at this time of the year can

409 be considered of little importance for cyanobacteria. In addition, in November the
410 metalimnion reaches greater depths (14-19 m in average) with limiting light intensities even
411 for a low-light adapted species such as *P. rubescens*. Indeed, *P. rubescens* moves to the upper
412 boundary of the metalimnion already in September due to light limitation in deeper zones
413 (Fig. 5).

414 Instead, the correlation of both, the start and end population of *P. rubescens* with the
415 metalimnion onset (Table 1), suggests that earlier formation is much more relevant for
416 population size than its termination. In addition, the correlation between population maximum
417 and decreasing average metalimnion thickness (Table 1) indicates that the reduced habitat size
418 is not (yet) a limiting factor for *P. rubescens* growth.

419 The strong context between biomass maxima and neutral buoyancy depth in June
420 clearly shows the behavioural adaptation of the *P. rubescens* population to still variable light
421 conditions during the initial phase of metalimnion formation. The cyanobacterium regulates
422 its depth within this zone through buoyancy according to daily insolation (Walsby et al.,
423 2001). Therefore, in the summer months (July-August), when the stratification in the lake is
424 stable, *P. rubescens* maxima are located in the zone of neutral buoyancy (Supplementary
425 Material, Fig. S5). This was already documented in previous studies showing close similarity
426 between neutral buoyancy depth and *P. rubescens* maxima during summer stratification
427 (Walsby & Schanz, 2002; Walsby et al., 2004). Although the reduction of its habitat size did
428 not seem to affect *P. rubescens* in July, it is nevertheless related to the depth of optimal
429 buoyancy: While the upper metalimnetic boundary decreased, the depth of neutral buoyancy -
430 exclusively related to ambient light conditions- did not change over time. This suggests that
431 the optimal zone for *P. rubescens* may come critically close to the mixed layer with
432 progressing lake warming. In September, both *P. rubescens* maxima and neutral buoyancy
433 depth were in critical vicinity to the upper boundary of the metalimnion (Fig. 5,
434 Supplementary Material, Fig. S5).

435 The main factor influencing *P. rubescens* biomass in summer was the biomass of the
436 preceding month. However, regression analysis revealed that temperature of the central
437 metalimnetic layer was also significantly correlated with cyanobacterial biomass, albeit only
438 in August. The average temperature of the metalimnion centre for July was 13 ± 1 °C between
439 1978 and 2013. The temperature in the centre did not increase significantly in July, but we
440 documented a long-term warming from 11.8 °C to 14.3 °C in August. Experiments with *P.*
441 *rubescens* cultures have shown that the growth rate increases with temperature, to a maximum
442 at 25 °C (Davis & Walsby, 2002; Oberhaus et al., 2007). Our findings, therefore, strongly
443 suggest that the warming of the metalimnion in late summer might have a positive effect on
444 the growth of *P. rubescens* at the rather stable optimal light (neutral buoyancy) conditions
445 during this period.
446
447 **Conclusion**
448 Our study identified significant shifts in onset and duration of the metalimnion and, moreover,
449 suggests an important role of this zone for transient heat storage in Lake Zurich. There are
450 indications that the compression and deepening of the metalimnion in late summer may
451 eventually restrict *P. rubescens* by forcing it into layers of unfavourable light conditions or
452 into the turbulent epilimnetic zone. However, the earlier onset and higher metalimnetic
453 temperatures might so far have more than counterbalanced potential negative effects as both
454 factors appear to favour the growth of *P. rubescens* in the studied system.
455
456 **Acknowledgements**
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458 Water Supply Zurich is also acknowledged for providing long-term data on *P. rubescens*. We
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461 University of Zurich.

462

463 All authors have declared no conflicts of interest.

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604 **Table 1**. Spearman's correlation analysis of *Planktothrix rubescens* start and end populations

605 (biomass between 0-20 m) versus metalimnion onset, duration and annual thickness.

	P. rubescens start (June) (tons)	*P. rubescens* end (November) (tons)
Onset (Julian days)	-0.43*	-0.38*
Duration (Julian days)	n.s.	n.s.
Annual thickness (m)	-	-0.48**

606 Significance levels: $p > 0.05$ (n.s.); $p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

607 **Figure legends**

608 **Fig. 1.** Temperature profiles, thermal gradients between 1 m depth layers and binary image of
609 the metalimnion exemplified for the years 1981 (**a**, **b** and **c**) and 2000 (**d**, **e** and **f**). Details on
610 the determination of metalimnion boundaries are described in the text.

611 **Fig. 2.** Temperature trends between 1978 and 2013. Left: analyzed for each month of the
612 year; significant changes ($p < 0.05$, MK trend test) in air (top) and water (bottom)
613 temperatures ($n = 36$ per depth per month). Color bar shows correlation range (*tau* values).
614 Seasonal MK trend test was applied for the whole year data-set for each depth. Right: Range
615 of water temperature increase per depth where significant trends were detected. Bars range
616 from minimum to maximum. Points and dotted line depict mean values. Rightmost column:
617 month, in which the maximum increase per depth was found.

618 **Fig. 3.** Temporal dimensions (a) and mean duration (b) of the metalimnion between 1978 and
619 2013. Bar color code: 1978-1989 (blue), 1990-1999 (yellow), 2000-2013 (red). Black line:
620 linear trend according to Sen's slope estimation with confidence intervals for stratification
621 onset (dashed) and end in (a), and duration in (b). $p < 0.01$ (**).

622 **Fig. 4.** Spatial dimensions of the metalimnion between 1978 and 2013. (a) upper boundary,
623 (b) lower boundary, (c) mean thickness. Bar colors: significant positive (red) or negative
624 (blue) trend over time according to MK trend tests. (d) MK trend analysis with Sen's slope of
625 decreasing mean thickness of the metalimnion for July-August ($p < 0.001$) with confidence
626 intervals.

627 **Fig. 5.** Mean depth of *P. rubescens* biomass maxima (orange line) and neutral buoyancy
628 (green line) between June and September. Gray area depicts the mean metalimnion
629 boundaries for each month, black dotted line shows the center of the metalimnion. Years in
630 which *P. rubescens* biomass was below detection limit are indicated by black triangles on the
631 x-axis.

632 **Fig. 6.** Relationship between *P. rubescens* biomass in June and July (a), July and August (b),
633 and between metalimnetic center temperatures (Tmc) and *P. rubescens* biomass in July (c)
634 and August (d). Points colors: 1978-1979 (black), 1980-1989 (green), 1990-1999 (blue),
635 2000-2013 (red). Development of Tmc (1978-2013) for July (e) and August (f) according to
636 MK trend analysis. Linear trend according to Sen's slope estimates. Significance levels: $p >$
637 0.05 (ns), $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***).

638 **Supplementary Material**

639 **Five supporting figures composed in one pdf file (Yankova_Supplementary_FiguresS1-**
640 **S5.pdf)**

641 S1. Long-term data of the biomass (µg fresh-weight L^{-1}) of the cyanobacterium *Planktothrix*
642 *rubescens* in Lake Zurich, Switzerland. Black lines in each year show the upper and lower
643 boundaries of the metalimnion during thermal stratification.

644 S2. Annual air temperature deviations from the mean for the period 1978-2013 for each
645 month. Black lines: trend according to Sen's slope estimation. Significance levels: $p < 0.05$
646 (*), $p < 0.01$ (**), $p < 0.001$ (***).

647 S3. Sen's slope coefficients for significant trends (see Fig. 2) of water temperature (lines and
648 points) and air temperature (dashed lines; yellow: November, pink: May, red: June, green:
649 April) between 1978 and 2013.

650 S4. Comparison between Schmidt stability (*S*) thresholds and metalimnion onset (a),
651 termination (b) and duration (c). Red dashed line marks the highest agreement between both
652 approaches.

653 S5. Bray-Curtis dissimilarity analysis for the parameters: upper metalimnion boundary
654 (Upper), lower metalimnion boundary (Lower), metalimnion center (Center), depth of
655 maximum *P. rubescens* (Max *P.r.*) and neutral buoyancy depth (NB).

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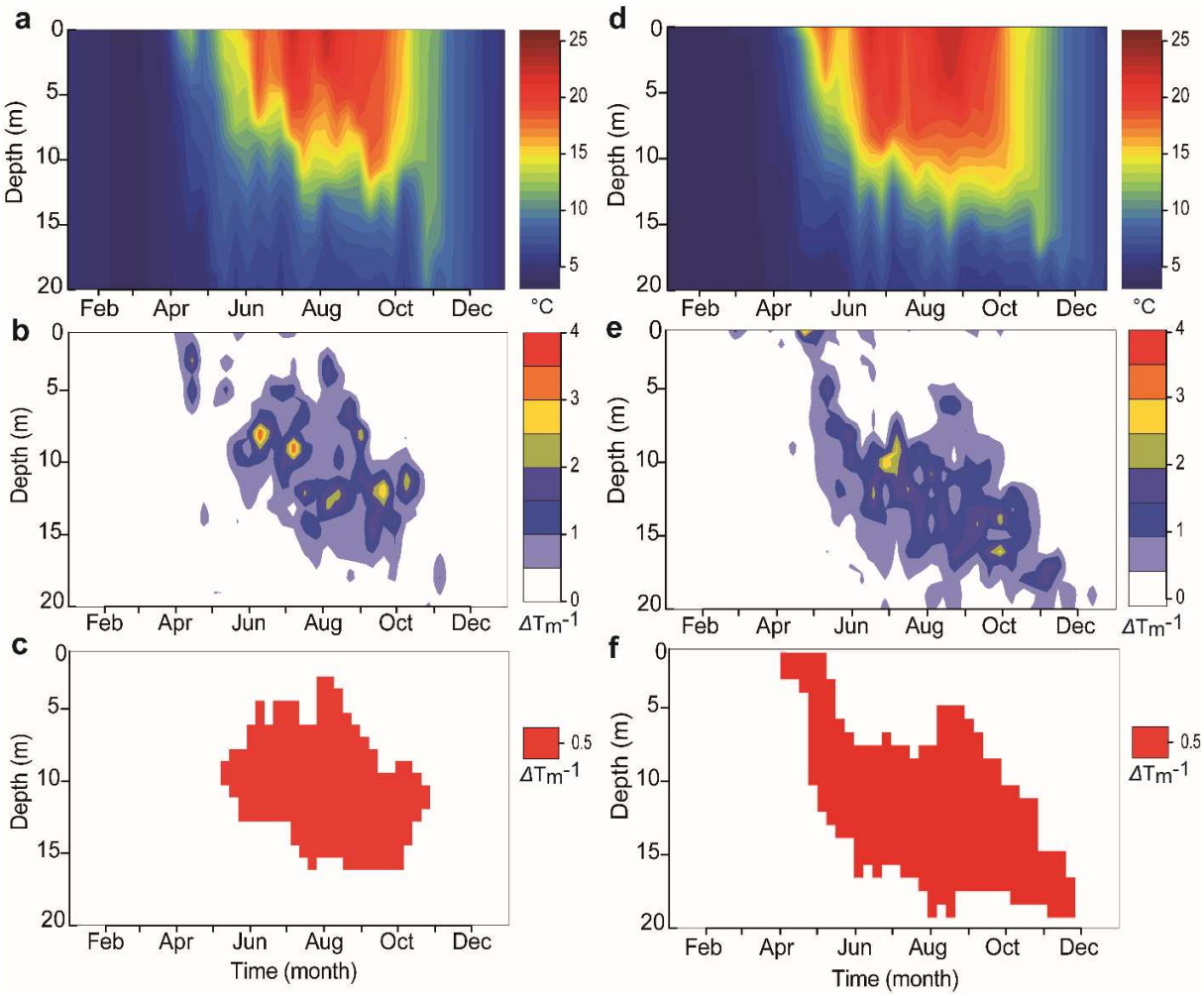
660 Fig. 1



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670 Fig. 2

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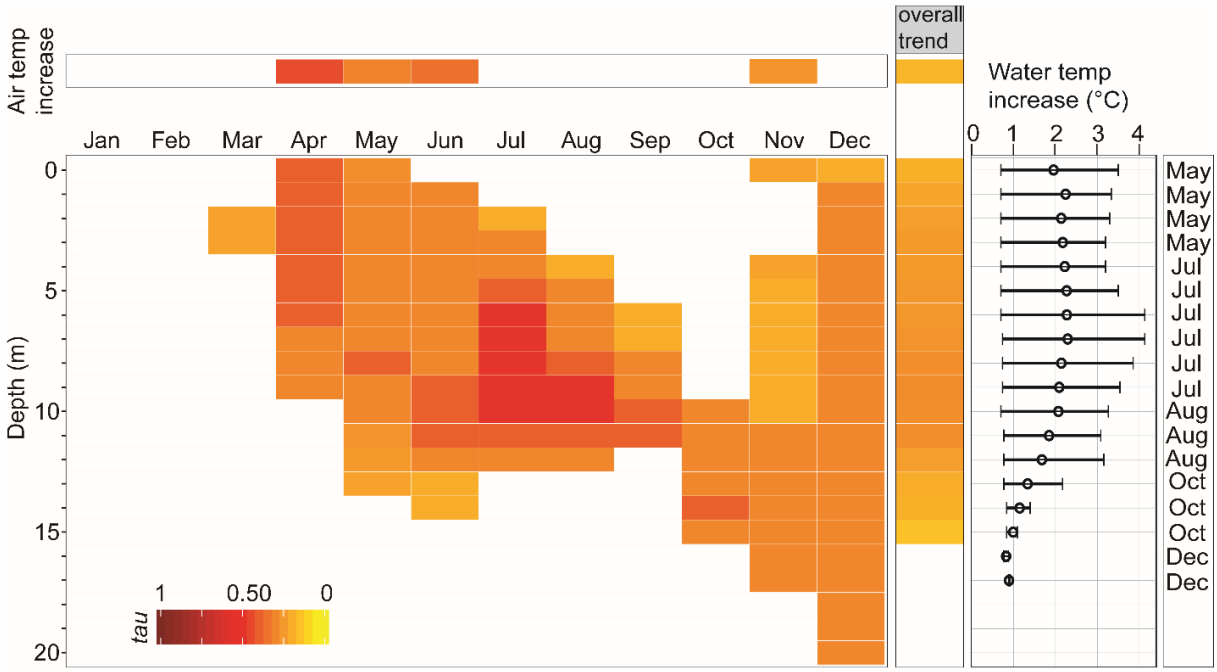

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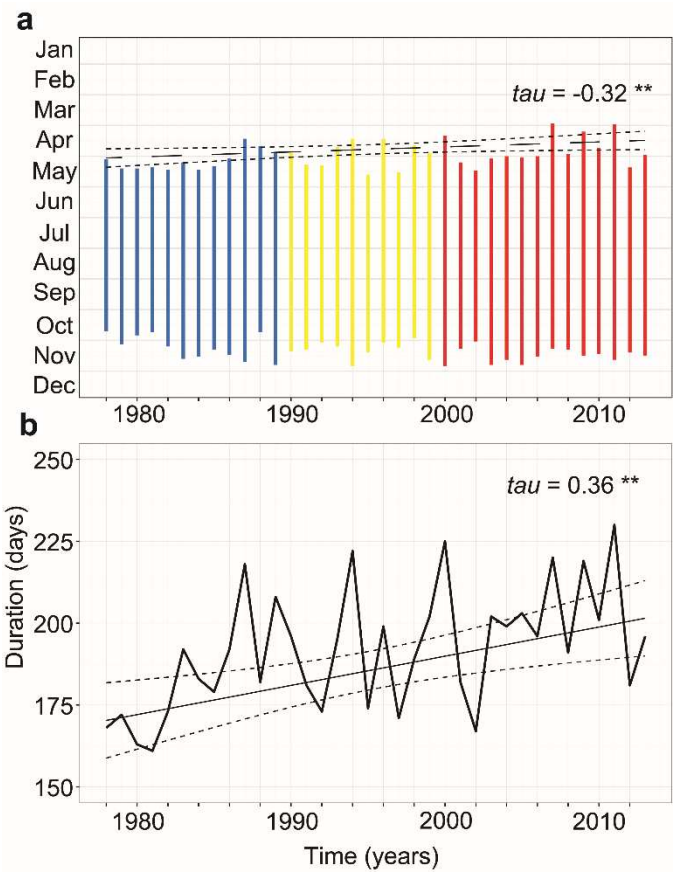
683 Fig. 3



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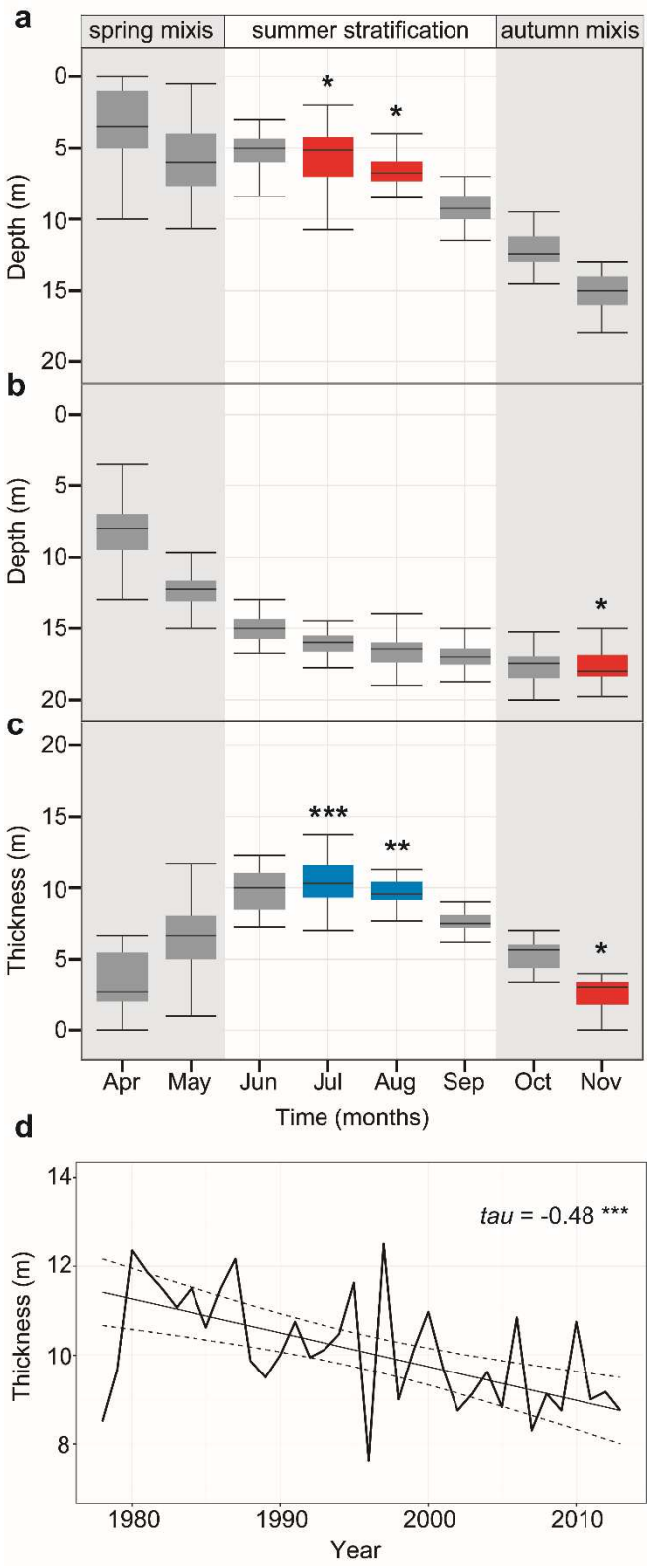
694 Fig. 4



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698 Fig. 5

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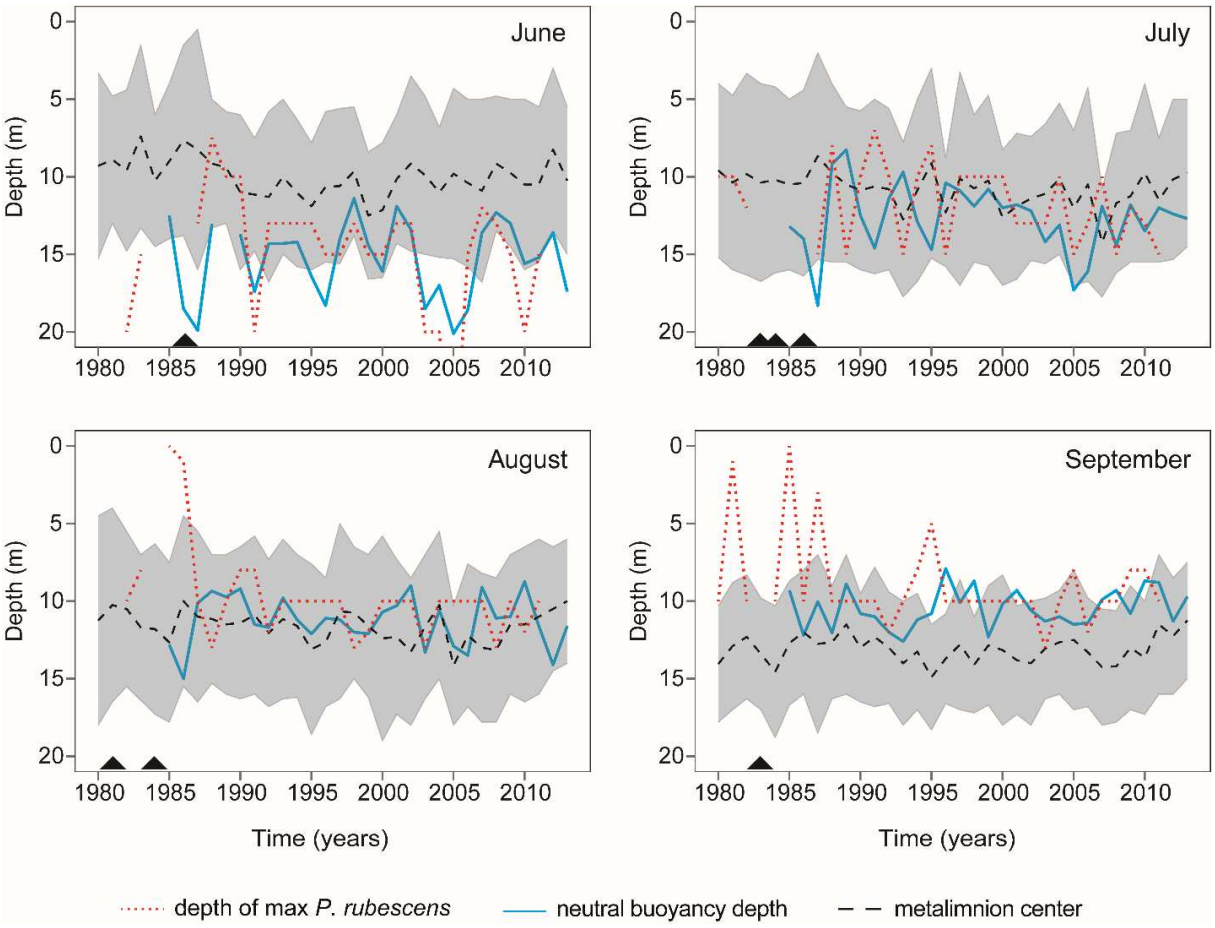

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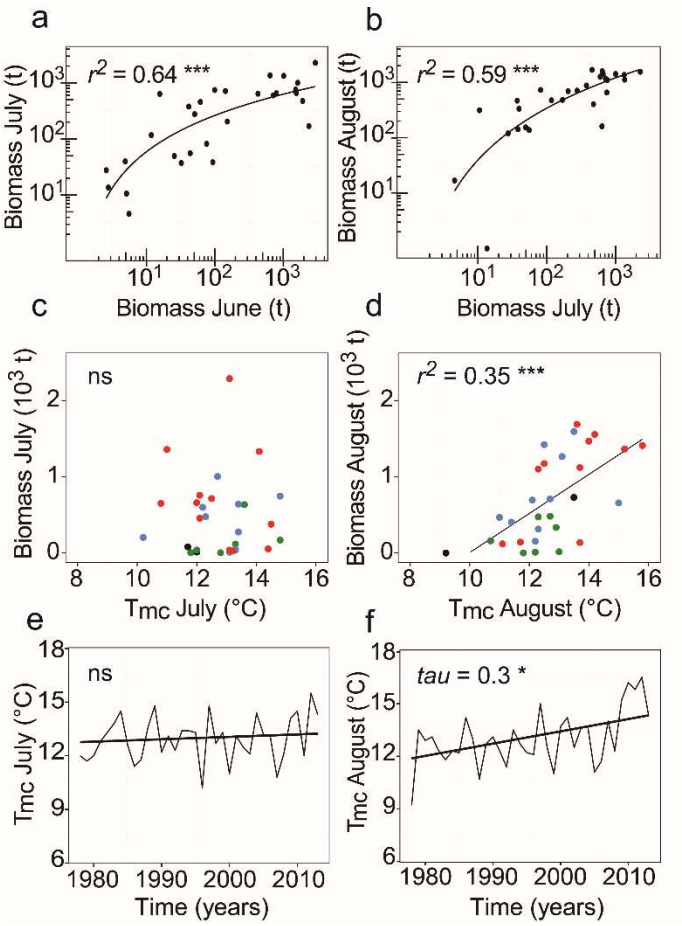
708 Fig. 6



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718 Fig. S1

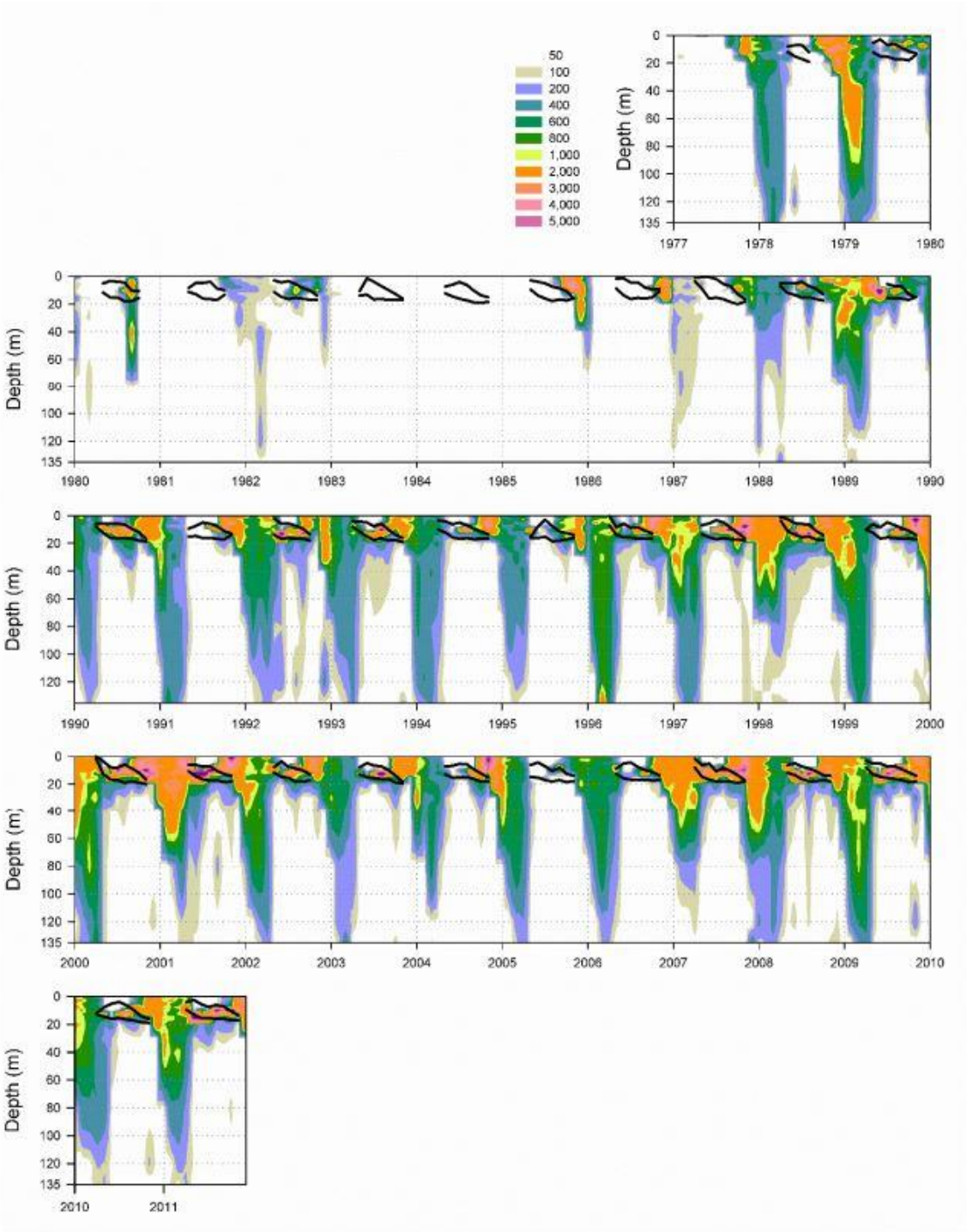

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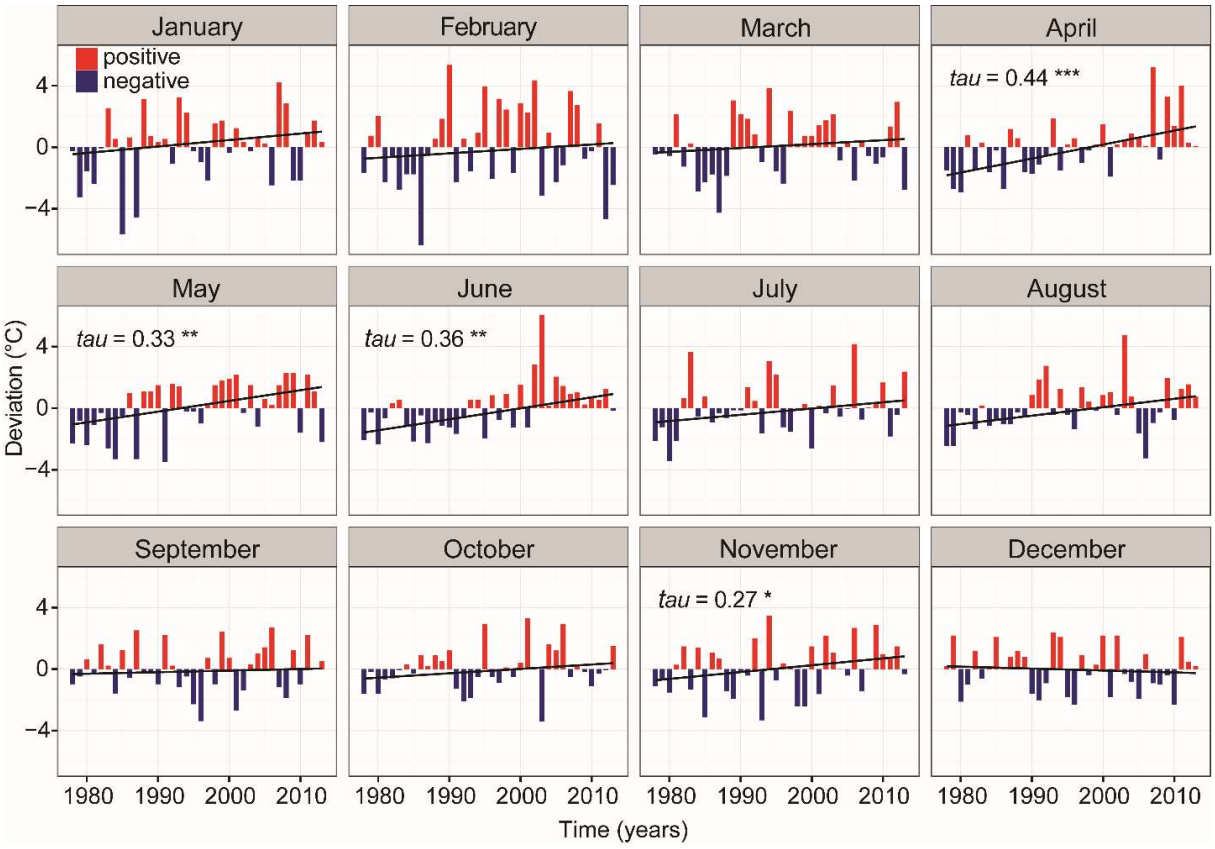
Fig. S2


January
positive
negative
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March
April
tau = 0.44 ***
May
tau = 0.33 **
June
tau = 0.36 **
July
August
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October
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tau = 0.27 *
December
Deviation (°C)
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−4
1980
1990
2000
2010
Time (years)

734 Fig. S3

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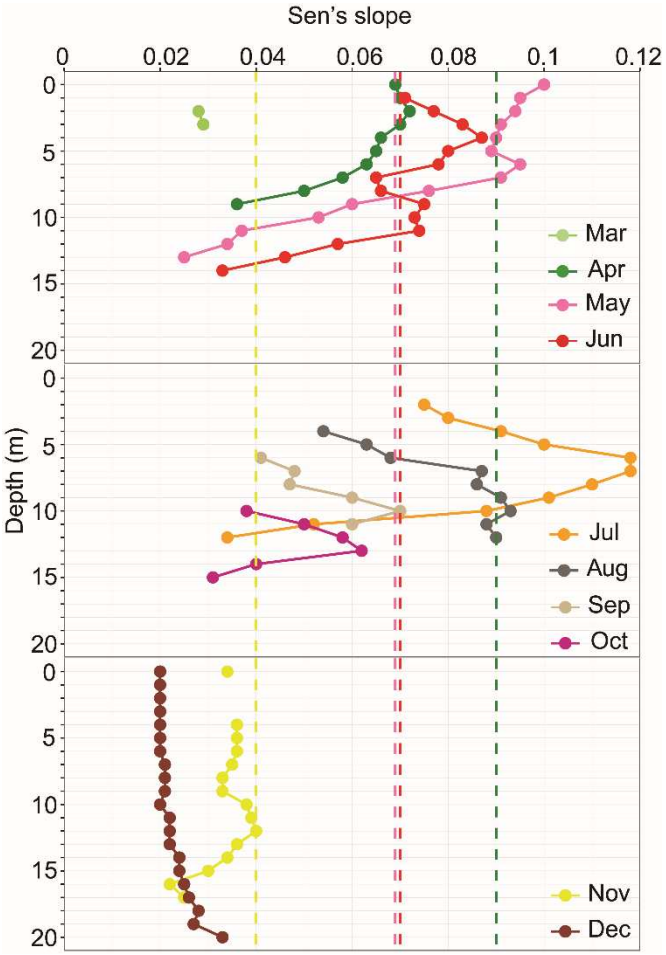

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744 Fig. S4

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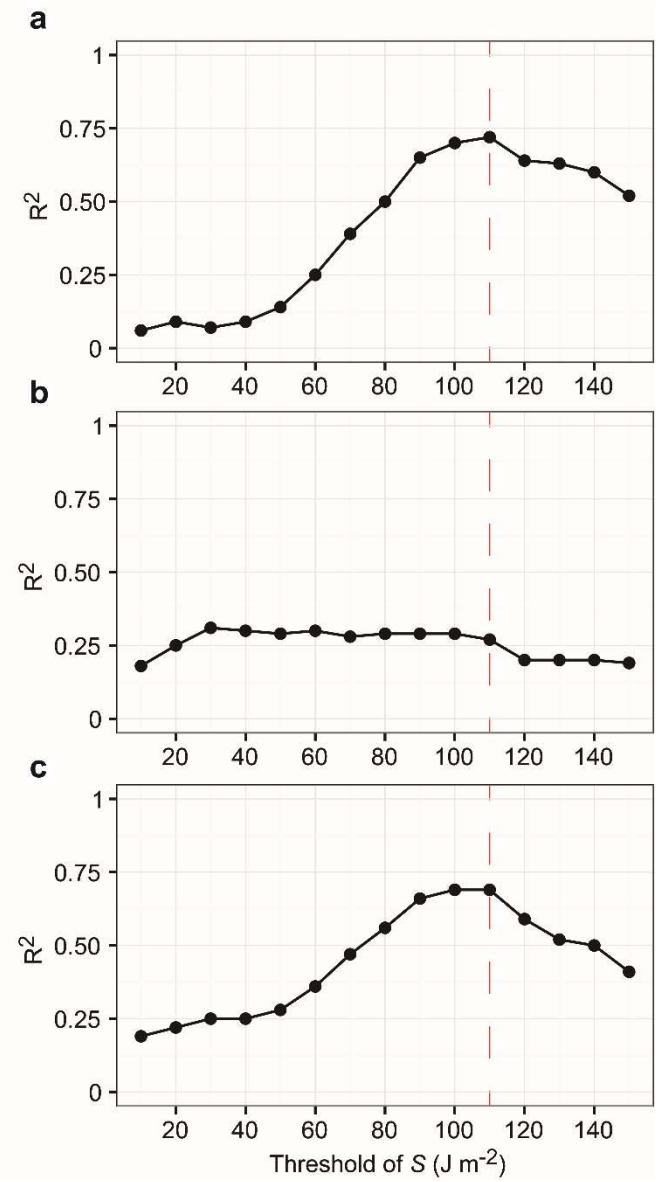

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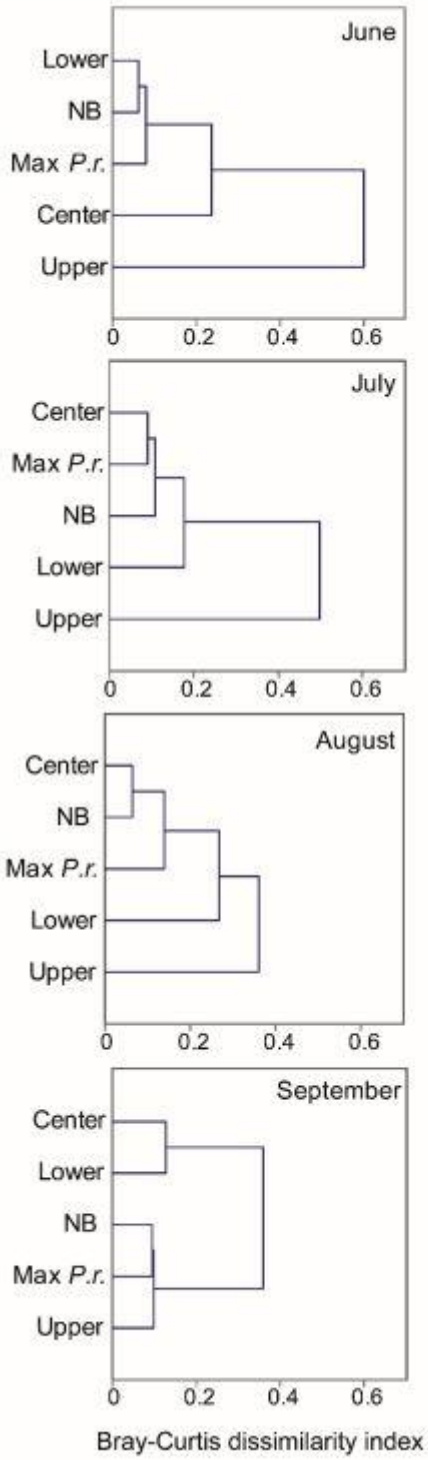
752 Fig. S5



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